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Developmental rate of *Rana* synkl. *esculenta* (Ranidae, Anura) embryos from different crosses: consequences on the evolution of the populations

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Crosses between different members of the *Rana* synkl. *esculenta* show a great variability concerning ontogenetic parameters. For this study developmental rate until stage 25 (GOSNER, 1960), v(25), and time interval to pass through this stage, dt(25), are used to measure differences between progeny of naturally observed combinations. For *esculenta* × *esculenta* progeny, mortality at this stage is very high; often all larvae of a cross die. Retardation of gene expression and nucleocytoplasmic incompatibility in early development could be the explanation of the differences in developmental rate. Mortality of *esculenta* homotypic crosses could be related to clone selection and not to accumulation of lethal genes : evolution by increasing variation and selection is not inhibited by reproduction without recombination. Clonally inherited genomes of *esculenta* that show high vitality in combination with *lessonae* genomes, give inviable progeny in homotypic crosses.

INTRODUCTION

European green frogs (*Rana* synkl. *esculenta* LINNÉ, 1758) (DUBOIS & GÜNTHER, 1982) are a very particular group of vertebrates. This group inludes two "good" species (*Rana ridibunda* Pallas, 1771 ; *Rana lessonae* Camerano, 1882) and their hybrid (*Rana* kl. *esculenta* Linné, 1758) (BERGER, 1967 ; TUNNER, 1973). *Rana* kl. *esculenta* remains in the hybrid state by a particular type of gametogenesis called hybridogenesis (TUNNER, 1974).

Consequently a frog with hybrid somatic cells produces gametes which are in their nucleotic and also in major part of their cytoplasmic component of parental type (generally *ridibunda*) (VOGEL & CHEN, 1976 ; GRAF, KARCH & MOREILLON, 1977) Nevertheless substances produced by somatic cells and transferred into the oocytes, like yolk, might have a hybrid nature and cause the differences observed between these eggs and eggs of the corresponding parental species concerning development and survival of progeny.

Green frogs populations not only contain individuals of different species, but also individuals of different ploidy levels. They can produce a great genetic variety in gametes and still a greater genetic variety in zygotes should be observed. But investigations on genotypes

of frogs found in natural populations show only certain genotypes, the others having been eliminated before or shortly after metamorphosis (GÜNTHER, 1983).

Crossing experiments among green frogs have shown an important mortality and a high number of anomalous larvae in certain combinations (BERGER, 1967 ; OGIELSKA-NOWAK, 1985). Developmental anomalies can be of two kinds : disturbances can be of short term for the population, like morphological anomalies of embryos and larvae and mortality at different developmental stages ; or they can be long term defaults modifying survival and fertility of adults.

Evolutionary success of asexual, clonal and hemiclonal species is doubtful (MULLER, 1932 ; MAYNARD SMITH, 1974). Asexual and clonal reproduction permits a quicker diffusion of an established genotype, but lowers variation and elimination of deleterious mutant alleles by default of recombination.

For this work I have chosen stage 25 (GOSNER, 1960), since larvae of this developmental stage are particularly affected in green frogs (BERGER, 1967 ; BLANKENHORN, HEUSSER & VOGEL, 1971 ; BINKERT, 1981). Ontogenetic events occurring in this stage seem to play an important part in the success of the different crossings. Similar developmental disturbances have been observed in green frog larvae from different populations all over Europe. Indications from these developmental patterns for evolution of hemiclonally reproducing *esculenta* will be discussed.

MATERIAL AND METHODS

The green frogs of this study came from eastern Austria (Seewinkel, Burgenland ; Lobau and Donauinsel, Vienna) and from central France (Brenne, Indre) (Table I). Parents of the crosses and tadpoles are kept in the collection of the Paris Muséum national d'Histoire naturelle (MNHN 1986.1763-1790) or in the author's collection.

Eggs were obtained from frogs without hormone injection so as to avoid damaging the eggs. Generally couples were taken in the natural environment (crosses number 1 – 10, 12 – 14 and 17 – 19) or reproductively active frogs were put together in the laboratory where they spontaneously amplected (crosses number 11, 15, 16 and 20). The whole clutches (900 to 3000 eggs) were transferred to indoor plastic containers (40 × 60 × 20 cm) and divided into portions of 20 to 30 eggs to assure oxygen supply. The embryos and larvae were reared at indoor temperature (water temperature : 18-22°C). About 300 small embryos were kept in a container. Development was controlled with a dissecting microscope and determined following the staging table of GOSNER (1960). When the larvae reached stage 25, the number of surviving larvae was limited to 50 by container (2 larvae / liter of water). They were fed a mixture of dried *Urtica dioica* and Tetra Min fish food. Dead and anomalous larvae were fixed in 4 % formalin.

Rate of development, v(25), is expressed as 100 divided by time in days to stage 25 (1 / T × 100) for the first embryo which reached this stage. This measurement gives the *optimal* development rate observed for a specific cross. The period during which stage 25 larvae could be observed, dt(25), is given in days. For every kind of crosses, the mean (m), the standard deviation (s) and the coefficient of variation following HALDANE (1955), V_H (DE-

LAUGERRE & DUBOIS, 1985), were calculated. The different crosses were compared by the non-parametric Mann-Whitney U-test (ELLIOTT, 1971). The correlation coefficient r was calculated with the statistical program package SPSSx (SCHUBÖ & UEHLINGER, 1986).

RESULTS

The two parental species differ in their developmental rates (Table I) (U-test : $U=0$; $n_1=4$; $n_2=4$; $p<0.05$). *Rana ridibunda* progeny takes a longer time to reach stage 25, but usually larvae get through this stage quickly. *Rana lessonae* embryos develop more quickly until stage 25, but a greater variation concerning the time requested to pass through this stage is observed.

Progeny of *esculenta* female × *esculenta* male shows a high variation in developmental rate ($m=8.30$; $s=1.78$; $n=6$; $V_H=21.45$) ; the extremes cover the developmental rates of progeny of *Rana ridibunda* (U-test : $U=9$; $n_1=4$; $n_2=6$; $p>0.05$) and *Rana lessonae* (U-test : $U=4$; $n_1=6$; $n_2=4$; $p>0.05$). The interval during which stage 25 larvae can be observed is very large.

Developmental rate of *lessonae* female by *esculenta* male embryos ($m-7.42$; $s=0.39$; $n=2$) resembles that of *Rana ridibunda* progeny ($m-7.16$; $s=0.42$; $n=4$), but stage 25 larvae are present during a much longer period. The progeny of the reciprocal cross (*esculenta* female × *lessonae* male) has a very high developmental rate ($m=10.07$; $s=1.01$; $n-3$) which is similar to that of *Rana lessonae* embryos ($m-10.33$; $s=0.98$; $n=4$). Here too the time interval during which stage 25 larvae are observed is very large.

At stage 25, especially in homotypic crosses of *esculenta* female × *esculenta* male, a high mortality of tadpoles was observed. All larvae of crosses (8) and (11) died ; only a few developed small buds of the hindlimbs. The larvae showed no particular anomalous features, but they were transparent and smaller than viable tadpoles of the same age. High lethality was observed only in *esculenta* homotypic crosses.

For green frogs from Austria, developmental rate is highly correlated with the date of reproduction ($r=0.61$; $f=14$; $p<0.01$). *Rana ridibunda*, which generally reproduces earlier in the year, has a lower developmental rate than *Rana lessonae* which is the latest of the green frogs to reproduce. In France, breeding of green frogs is earlier in the year than in Austria, but developmental rates of the crossings correspond to what was observed in Austrian larvae.

DISCUSSION

Stage 25 in anuran development is the product of embryogenesis and the starting point for larval development which gives the basis of parameters at metamorphosis (age, size) (WILBUR & COLLINS, 1973 ; TRAVIS, 1981). In the beginning stages, development depends on maternal factors stocked in the oocyte. Only in late blastula stages are paternal genes expressed (WRIGHT & MOYER, 1968). This is often a critical point in hybrid ontogeny and various disturbances can be observed : development stops in these stages or expression of pa-

Table I. – Developmental rate of different crosses within *Rana* synkl. *esculenta*. (L, localities : V, Vienna ; B, Burgenland ; I, Indre ; date, date when eggs were laid ; M, male ; F, female ; v(25), rate of development ; I(25), age interval in days of stage 25 larvae ; dt(25), time interval during which stage 25 (GOSNER, 1960) tadpoles were observed ; R, *Rana ridibunda* ; E, *Rana* kl. *esculenta* ; L, *Rana lessonae* ; m, mean ; s, standard deviation ; V_H, HALDANE coefficient of variation).

Cross	L	Date	M	F	v(25)	I(25)	dt(25)
(1)	V	11.5.1983	R	R	7.69	13 - 15	2
(2)	V	11.5.1983	R	R	6.67	15 - 17	2
(3)	V	11.5.1983	R	R	7.14	14 - 26	12
(4)	V	6.5.1984	R	R	7.14	14 - 16	2
					m=7.16		m=4.50
					s=0.42		s=5.00
					V_H=5.87		V_H=111.11
(5)	V	28.4.1983	R	E	5.26	19 - 81	62
(6)	B	28.5.1983	E	E	6.67	15 - 49	34
(7)	B	28.5.1983	E	E	9.09	11 - 59	48
(8)	B	28.5.1983	E	E	11.11	9 - 18	9
(9)	B	6.6.1984	E	E	7.14	14 - 27	13
(10)	B	17 6.1984	E	E	9 09	11 - 16	5
(11)	I	4.5.1986	E	E	6.67	15 - 50	35
					m=8 30		m=24.00
					s=1.78		s=17.33
					V_H=21.45		V_H 72.26
(12)	B	28.5.1983	L	E	10.00	10 - 35	25
(13)	B	28.5 1983	L	E	9 09	11 - 56	45
(14)	B	28 5.1983	L	E	11.11	9 - 23	14
					m=10.07		m=28.00
					s=1 01		s=15.72
					V_H=10.05		V_H=56.13
(15)	I	5.5.1986	E	L	7.69	13 - 50	37
(16)	I	7.5.1986	E	L	7.14	14 - 49	35
					m 7 42		m 36.00
					s=0.39		s=1.41
					V_H 5 24		V_H 3.93
(17)	B	31.5.1983	L	L	11.11	9 - 23	14
(18)	B	31.5.1983	L	L	9.09	11 - 47	36
(19)	B	31.5.1983	L	L	11.11	9 - 17	8
(20)	I	8.5.1986	L	L	10.00	10 - 34	24
					m=10.33		m=20.50
					s=0.98		s=12.26
					V_H 9.46		V_H=59.80

ternal genes is delayed or inhibited (WHITT, CHO & CHILDERS, 1972) ; morphological anomalies (e.g. exogastrulae : DELARUE et al., 1985 ; ELINSON, 1981 ; HENNEN, 1963 ; HERTWIG, RUHLAND & WEISS, 1958) can be the epigenetic reflection of these disturbances. Usually the embryonal phase ends without new disturbances, but anomalous embryos can be observed in tailbud stage or later ; these morphological disturbances should be regarded as a consequence of difficulties in the gastrula phase. In stage 25 transition to active larval life takes place, which includes physiological modifications (nutrition).

Different time intervals required to reach stage 25 (Table I) reflect events in embryonic development (retardations, etc.). Variation in the time necessary to pass through stage 25 (Table I) can be related to hybrid genome constitution of *esculenta* progeny and might therefore reflect developmental difficulties in the transition from embryonic to larval life. In *lessonae* larvae it might rather be a consequence of genetic variation, perhaps due to lack of competition with *esculenta* larvae in mixed populations.

In the hybridogenetic taxon *esculenta*, gametogenesis results in oocytes with a non-recombined genome which consists entirely of chromosomes of one parental type (*ridibunda* : VOGEL & CHEN, 1976 ; GRAF, KARCH & MOREILLON, 1977 ; or *lessonae* : GÜNTHER 1983 ; BERGER & GÜNTHER, 1988). To a large extent, their cytoplasm resembles that of the oocytes of this parental species.

Zygotes produced by homotypic *esculenta* crosses have been shown to contain two sets of the same parental genome and a corresponding cytoplasm, like in homotypic crosses of the parental species. But the genomes did not pass through recombination. On the other hand, when *esculenta* eggs are fertilized by *lessonae* sperm, the resulting zygotes have a hybrid genome and a cytoplasm of *ridibunda* type. This could appear to be a rather unbalanced situation, but in fact this combination is very successful.

The observations on different crosses of green frogs can be studied in the light of their cytogenetic constitution. The rather homogeneous developmental rate of the crosses where parental species are involved (Table I) is interpreted as a consequence of recombination and gene flow in these populations. Only *ridibunda* tadpoles get through stage 25 very quickly (Table I). In *lessonae* crosses, tadpoles usually have delayed development at these stages. On the other hand, the variation in developmental rate in *esculenta* homotypic crosses has been interpreted as a consequence of clonal inheritance of the genomes in the involved taxon which should lead to accumulation of deleterious genes (BERGER, 1976 ; GRAF & MÜLLER, 1979 ; HOTZ, 1983 ; BINKERT, BORNER & CHEN, 1982). Embryos of these crosses are also affected by various morphological anomalies ("*esculenta* developmental syndrom", OGIELSKA-NOWAK, 1985 ; OHLER, 1987).

Only in homotypic *esculenta* crosses, the developmental disturbances can be lethal for all larvae within a cross. These lethal crosses are very numerous in populations where *lessonae* and *esculenta* live in sympatry and where *esculenta* entirely depends upon *lessonae* for its reproduction, whereas they are less important in populations where *lessonae* is absent. In the latter, the genetical basis for hybrid state conservation is different, and triploid *esculenta* play an important role in producing gametes of *lessonae* type (GÜNTHER, 1983).

Very similar developmental phenomena concerning homotypic *esculenta* crosses are observed in geographically distant populations (BERGER, 1967 ; BLANKENHORN, HEUSSER & VOGEL, 1971 ; GÜNTHER, 1973 ; OHLER, 1987 ; TUNNER, 1979, 1980 ; WIJNANDS, 1979). If

they are related to genetic load of *esculenta* hemiclones, it would mean a very wide distribution of these lineages. Also, genetic load does not seem to be superior in green frogs from clonal lineages where recent hybridization can be excluded (HOTZ, 1983). Various hypotheses about evolutionary dead-end or success of clonally (asexual) reproducing species have been or can be proposed ; particularly two of them are worthy of discussion : MULLER's ratchet mechanism and a model of evolution in asexual populations.

MULLER's ratchet mechanism (1932) explains that in asexually reproducing species the load of mutations cannot decrease below that already present in the least loaded clone, but that it can increase. In consequence, mutations, that should be mostly deleterious ones, will accumulate in clonally inherited genomes and such lineages are rather condemned to extinction over long term evolution.

This is true within a clone, but not for a clonally reproducing "species" which includes a series of different clones. There are two sources for the variation among clones : multiple origins and mutation (ANGUS & SCHULTZ, 1979). Recombination provides a much greater variation than mutation alone can do. This is the major advantage of sexual reproduction. It accelerates evolution very substantially, particularly in large populations (MAYNARD SMITH, 1974). But in sexually reproducing species a given genotype is lost for the next generation and is unlikely to reappear. Clonal inheritance of the genome prevents the loss of a certain combination of genes. This can be favorable despite the shortage of variation, for example in small colonizing populations. Asexual reproduction can assure the rapid increase of a given genotype. As shown by mathematical models, evolution is not stopped by asexual reproduction, but evolution rate may be reduced under certain conditions (MAYNARD SMITH, 1974).

Variation in *esculenta* clonal lineages comes from primary hybridization in areas where the two parental species occur in sympatry, and from mutation. There is still an other possibility for increasing variation in regions where only *lessonae* and *esculenta* occur : some of the progeny of *esculenta* homotypic crosses can survive to sexual maturity ; these individuals may have *ridibunda* genotypes and can act as founders of new hybridogenetic lineages.

The very similar developmental pattern of *esculenta* × *esculenta* germs from populations of all over Europe reflects rather a cytoplasmic inconvenience or an incompatibility of regulatory genes than an accidental accumulation of lethal genes and their subsequent dispersion in hemiclonal lineages. In regions where primary hybridization can still occur, *esculenta* × *esculenta* crosses show a high variability in developmental pattern (BERGER, 1967). On the other hand, the variability of developmental pattern is reduced where primary hybridization founded clonal lineages long time ago (HOTZ, 1983). The different *esculenta* lineages will be selected that assure a successful development in *esculenta* × *lessonae* crossings and that assure the maintenance of the hybrid.

Lethality at stage 25 in *esculenta* homotypic crosses means an important loss of gametes for the population. But as these germs die before feeding they don't compete with vital larvae of the other combinations concerning food, and also space (crowding). As their death coincidates with the starting of feeding of the vital larvae and as green frog larvae may be cannibalistic, especially on feeble tadpoles, they could be a source of food, and their unsuccessful development would allow the transmission to these larvae of a great part of the substances of the *esculenta* oocytes.

To estimate the influence of life-history parameters of a population, the combination

of a series of facts must be considered. The loss of gametes by *esculenta* homotypic crosses can be leveled out by the subsequent consumption of the larvae by the other members of the population. This supplementary food source should favour their larval development and metamorphosis.

Hybrid embryos of *esculenta* type (from *esculenta* × *lessonae* crossings) are as successful as *lessonae* larvae in developmental rate and in passing through stage 25. In E-L populations, larvae of the two types have equal ontogenetic potential. Developmental success of larvae from homotypic *esculenta* crosses is extremely reduced. Nevertheless, the *esculenta* genetic system seems very stabilized, which permits the large radiation observed in these frogs. The maintenance of *Rana* kl. *esculenta* is assured by cytoplasmic compatibility in hybrid zygotes, success of larval development and a variety of genetic mechanisms for meiosis in different population systems.

RÉSUMÉ

Les produits des croisements réalisés entre différents membres du synklepton de *Rana* kl. *esculenta* manifestent une grande variabilité en ce qui concerne leurs paramètres ontogénétiques. Pour cette étude, deux indices, le taux de développement jusqu'au stade 25 de GOSNER (1960), v(25), et la durée de l'intervalle nécessaire pour traverser ce stade, dt(25), ont été utilisés pour mesurer les différences entre les descendances obtenues à partir de diverses combinaisons parentales observées dans la nature. La mortalité au stade 25 est très élevée dans la descendance issue des croisements homotypiques *esculenta* × *esculenta* : souvent toutes les larves d'un croisement meurent. Ces différences développementales pourraient être dues à des retardations dans l'expression de certains gènes et des incompatibilités nucléocytoplasmiques au début du développement. La mortalité observée dans les croisements homotypiques *esculenta* pourrait être due à la sélection des clones et non pas à l'accumulation des gènes léthaux : l'évolution par augmentation de la variation et sélection n'est pas supprimée par la reproduction sans recombinaison. Les génomes *esculenta* transmis de manière clonale qui montrent une vitalité élevée en combinaison avec les génomes *lessonae*, donnent une descendance inviable dans les croisements homotypiques.

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